REMARKS

Claims 3 and 7 are amended to depend from claim 18. Support for these amendments can be found at, for example, page 19, lines 6-19 of the specification. Claim 12 is amended. Support for this amendment can be found at, for example, page 24, lines 1-6 of the specification. Claim 14 is amended. Support for this amendment can be found at, for example, page 10, lines 10-18 of the specification.

Claim 15 is newly added. Support for this amendment can be found at, for example, page 16, line 13 through page 19, line 5 of the specification.

Claim 16 is newly added. Support for this amendment can be found at, for example, page 36, lines 3-5 of the specification.

Claim 17 is newly added. Support for this amendment can be found at, for example, page 17, lines 16-17 of the specification.

Claim 18 is newly added. Support for this amendment can be found at, for example, page 19, lines 6-11 of the specification.

Claim 19 is newly added. Support for this amendment can be found at, for example, page 19, lines 15-19 of the specification.

Claim 20 is newly added. Support for this amendment can be found at, for example, page 19, lines 20-25 of the specification.

Claim 21 is newly added. Support for this amendment can be found at, for example, page 20, lines 1-5 of the specification.

Claim 22 is newly added. Support for this amendment can be found at, for example, page 20, lines 10-16 of the specification.

Claim 23 is newly added. Support for this amendment can be found at, for example, page 20, lines 11-21 of the specification.

Claim 24 is newly added. Support for this amendment can be found at, for example, page 21, lines 7-16; page 21, lines 22-25, and page 22, lines 1-4 of the specification.

Claim 25 is newly added. Support for this amendment can be found at, for example, page 24, lines 11-22 and page 23, lines 19-25 of the specification.

Claim 26 is newly added. Support for this amendment can be found at, for example, page 17, lines 4-10 of the specification.

Claim 27 is newly added. Support for this amendment can be found at, for example, page 8, line 17 of the specification.

Claims 1, 2, 4-6, 8-11, and 13 are canceled.

Rejection under 35 U.S.C. § 103

On pages 2-7 of the Office Action, claims 1-14 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Tsugita et al. (Electrophoresis, 1998, Vol. 19, pages 928-938) ("Ref. 1: Tsugita et al") in view of Tsugita et al. (Chemistry Letters, 1992, pages 235-238) ("Ref. 2: Tsugita et al 1992") and Vogt et al. (Polymer Bullentin, 1996, Vol. 36, pages 549-555) ("Ref. 3: Vogt et al").

Applicants' Response

In response, Applicants respectfully submit that the presently claimed invention is not rendered obvious by any combination of the cited references, as asserted below.

Regarding Reference 1: Tsugita et al.

Ref. 1: Tsugita et al. teaches such a process for C-terminal sequencing for the protein, which process comprises following steps:

The first step of extracting the protein from the protein spot on the polyacrylamide gel is carried out by using the following extraction procedure. See Abstract of Ref. 1: Tsugita et al.

The protein spot was excised from the polyacrylamide gel and broken up by the use of a small hand-held homogenizer after addition to the 500 μL of 6M guanidine-HCl, 0.1% SDS, 0.5 M Bicine, 4mM EDTA, pH 8.0-8.5. The 6M guanidine-HCl and 0.1% SDS contained in the solution is successfully used to denature the protein, and thus the denatured protein can be easily extracted from the gel carrier to be collect in the pool of supernatant and two addition washes. See page 930, column 2, paragraph 3 of Ref. 1: Tsugita et al.

The denatured protein contained in the pooled supernatant is subjected to purification and separation with use of a mini-column of C18 silica. The denatured protein isolated by the column separation method is dried up to use as the dried protein sample.

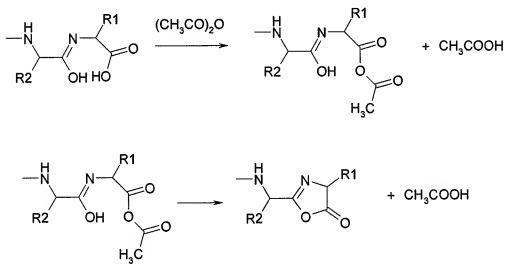
Therefore, Ref. 1: Tsugita et al. fails to teach any process for C-terminal sequencing, in which the reactions for C-terminal stepwise degradation are carried out for the peptide being maintained in a state that it is bound on the gel carrier.

Ref. 1: Tsugita et al. teaches such a procedure of reactions for C-terminal stepwise degradation comprising the following three reaction sub-steps (i) – (iii):

(i) The first reaction sub-step for acetylation of the N-terminus of the peptide (denatured protein) and formation of an oxazolone at the C-terminal carboxyl group of the peptide (denatured protein):

Acetic anhydride with 20% acetic acid tetrahydrofuran solution in the present of 1% DTT was reacted on the dried sample of peptide (denatured protein) at 60 °C for 10 min. See page 930, column 2, paragraph 3 of Ref. 1: Tsugita et al. The reaction of formation of the oxazolone may be carried out by the following reaction scheme:

Formation of the oxazolone at the C-terminal carboxyl group:



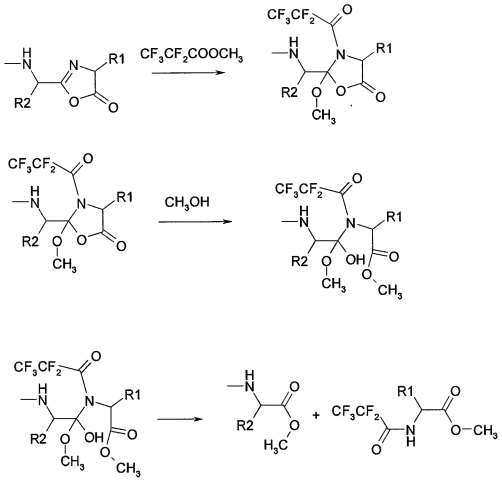
The acetic acid may be used as a catalyst for inducing the convention of keto-form of the amido moiety into the enol-form.

(ii) The second reaction sub-step for degradation of the oxazolone-ring to liberate the C-terminal amino acid and to form the esterified peptide:

The reaction is made with 5% PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3) in methanol (CH_3OH) at 5 °C for 15 min. See page 930, column 2, paragraph 3 of Ref. 1: Tsugita et al.

The reaction of degradation of the oxazolone may be carried out by the following reaction scheme:

Degradation of the oxazolone:

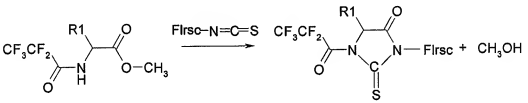


The C-terminal amino acid was liberated in the form of N-(pentafluoropropanoyl) amino acidic methyl ester, and thus, the peptidyl reaction product was formed in the shape of esterified peptide.

Therefore, PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3) was used as a reactant for the addition reaction on the double bond of >C=N- type.

The C-terminal amino acid that was liberated in the form of N-(pentafluoropropanoyl) amino acidic methyl ester was isolated from the esterified peptide, and then, the C-terminal amino acid (N-(pentafluoropropanoyl) amino acidic methyl ester) isolated was modified with fluorescien isothiocyanate and analyzed by HPLC. See page 931, column 1, paragraph 1 of Ref. 1: Tsugita et al.

Modification with fluorescien isothiocyanate (Flrsc-N=C=S):



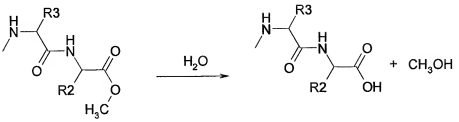
On the other hand, the esterified peptide collected from the reaction solution was subjected to the final reaction.

(iii) The final reaction sub-step for conversion of the esterified peptide (peptidyl methyl ester) into the peptide with a free carboxyl group at its C-terminus:

10 % DMAE aqueous solution was used at 60 °C for 20 min in the hydrolysis reaction of the ester bond to convert the esterified peptide (peptidyl methyl ester) into the peptide with a free carboxyl group. See page 930, column 2, paragraph 3 of Ref. 1: Tsugita et al.

The reaction of hydrolysis of ester may be carried out by the following reaction scheme:

Hydrolysis of ester:



The peptide with a free carboxyl group was collected from the aqueous solution, and then was dried up to use as a dried peptide sample for the next degradation step.

Accordingly, the peptidyl reaction product of the above process (peptide with a free carboxyl group), which is obtained in each of the C-terminal degradation steps, is by no means analyzed by mass spectroscopy. See page 931, column 2, paragraph 1 of Ref. 1: Tsugita et al.

Indeed, the C-terminal sequence of the denatured protein was made based on the HPLC analysis of the C-terminal amino acid (N-(pentafluoropropanoyl) amino acidic methyl ester) obtained in the sub-step (ii). See page 931, column 1, paragraph 1 of Ref. 1: Tsugita et al.

Therefore, the dried peptide sample, which has the C-terminal amino acid of –NH-CH(**R2**)-COOH to be analyzed in the next step of the C-terminal degradation, should be free from such contamination of the denatured protein that retains un-reacted C-terminal amino acid of –NH-CH(**R1**)-COOH.

At least, Ref. 1: Tsugita et al. fails to teach any process for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

Further, Ref. 1: Tsugita et al. also teaches another process for multi-point C-terminal sequencing (i.e. Chemical specific cleavage and multiple C-terminal sequencing) for the protein, where the process is carried out on the dried protein as well as on the polyacrylamide gel.

The process for the multi-point C-terminal sequencing for the protein sample on the polyacrylamide gel comprises the following steps (a) - (c):

(a) Step of electro-blotting the protein on the polyacrylamide gel to the Immobilon-CD membrane:

At first, the proteins were subjected to one-dimensional or two dimensional electrophoresis on the polyacrylamide gel. The resulted protein spots on the polyacrylamide gel were electroblotted to the Immobilon-CD membrane and negatively strained. The protein spot identified on the Immobilon-CD membrane was excised and cut into a 1 mm square. See page 931, column 2, paragraph 1 of Ref. 1: Tsugita et al.

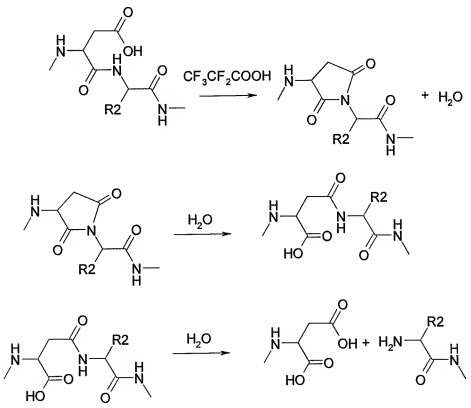
(b) Step of chemical specific cleavage of protein on the blotted membrane :

The cut-off square peace of the blotted membrane was put in the small tube and subjected to the specified cleavage reactions. In the specified cleavage reactions, the protein sample was cleaved at the carboxyl side of the aspartyl pepdide bond (Asp-C), or at the amino side of the serine or threonine (Ser/Thr-N) peptide bonds, under the specified cleavage conditions, respectively. See page 931, column 2, paragraph 1 of Ref. 1: Tsugita et al.

Ref. 1: Tsugita et al. employed such a specified cleavage condition for the Asp-C cleavage reaction that a vapor phase reaction was made with a **vapor** generated from a 0.2 % PFPA aqueous solution containing 1 % w/v DTT at 90 °C for 4-16 h. See page 931, Figure 1, caption of Ref. 1: Tsugita et al.

The group of peptidyl reaction products (peptide fragments) produced in the reaction of cleavage at the carboxyl side of the aspartyl pepdide bond (Asp-C) will consist of the N-terminal peptide fragment having a newly exposed C-terminal aspartic acid, inner peptide fragments having a newly exposed C-terminal aspartic acid and the C-terminal peptide fragment. See page 932, column 1, paragraphs 1-3 of Ref. 1: Tsugita et al.

The Asp-C cleavage reaction may be made through the following reaction scheme.

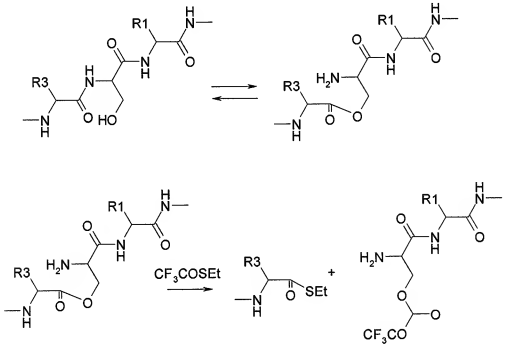


Ref. 1: Tsugita et al. employed such a specified cleavage condition for the See/Thr-N cleavage reaction that a vapor phase reaction was made with a **vapor** of TFASEt (S-Ethyl

trifluorothioacetate; $CF_3CO\text{-}S\text{-}CH_2CH_3$) at 30 ℃ for 24 h or at 50 ℃ for 6-24 h. See page 931, Figure 1, caption of Ref. 1: Tsugita et al.

The group of peptidyl reaction products (peptide fragments) produced in the reaction of cleavage at the amino side of the serine or threonine (Ser/Thr-N) peptide bonds will consist of the N-terminal peptide fragment, inner peptide fragments having a newly exposed N-terminal Ser/Thr residue and the C-terminal peptide fragment having a newly exposed N-terminal Ser/Thr residue. See page 932, column 1, paragraph 4 and column 2, paragraph 1 of Ref. 1: Tsugita et al.

The Ser/Thr-N cleavage reaction may be made through the following reaction scheme.



(c) Step of extraction of the peptidyl reaction products from the cut-off square peace of the membrane.

After the specified cleavage reaction, the peptidyl reaction products (peptide fragments) were extracted with 30% and 60% acetonitrile aqueous solutions. The extract was dried and

analyzed by FAB-MS or MALDI-TOF-MS. See page 932, column 2, paragraph 2 of Ref. 1: Tsugita et al.

However, Ref. 1: Tsugita et al. fails to teach any process in which chemical specific cleavage was carried out on the protein being maintained in a state that it is bound on the polyacrylamide gel in place of the protein on the blotted membrane.

In view of these facts, Ref. 1: Tsugita et al. fails to provide any suggestion as to such a process for C-terminal stepwise degradation or for chemical specific cleavage, which is carried out on the peptide (denatured protein) being maintained in a state that it is bound on the gel carrier such as polyacrylamide gel.

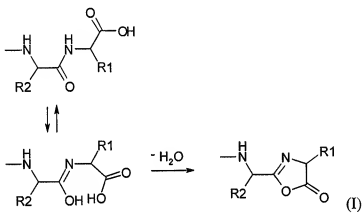
Ref. 1:Tsugita et al. fails to provide any suggestion as to such a process for C-terminal stepwise degradation, which is suitable used for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

Further, Ref. 1:Tsugita et al. fails to provide any suggestion as to whether or not PFPA, which is suitably used in the vapor phase reaction for the Asp-C cleavage reaction, would be employed as a reactant for the liquid phase reaction for degradation of the oxazolone-ring, in place of PFPMe. At least, the function of PFPMe used in the liquid phase reaction for degradation of the oxazolone-ring is quite different from the catalytic function of PFPA used in the vapor phase reaction for the Asp-C cleavage reaction. Therefore, there is no good reason to believe that PFPA would have a similar function to that of PFPMe used in the liquid phase reaction for degradation of the oxazolone-ring.

In contrast, the process for releasing the C-terminal amino acids successively from the peptide of the present invention is carried out through the following reaction schemes:

(I) reaction for formation of 5-oxazolone ring:

The reaction for formation of 5-oxazolone ring is expressed on the whole by the following reaction scheme (I):



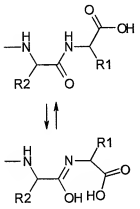
(I)

See page 32, lines 8-13 of the specification.

The reaction of scheme (I) consists of the following two stages (Ia) and (Ib).

(Ia) keto-enol tautomerism:

The perfluoroalkanoic acid contained in the mixed solution of the alkanoic acid anhydride and the perfluoroalkanoic acid dissolved in the dipolar aprotic solvent is allowed to act as a proton donor on the dried peptide at the stage of keto-enol tautomerism, as shown in the following reation scheme (Ia):

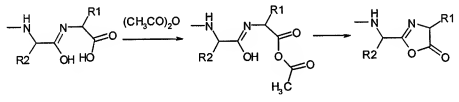


(Ia).

See page 32, line 8 through page 33, line 5 of the specification.

(Ib) formation of the activated C-terminal carboxyl group and formation of the intramoecular ester bond (formation of the 5-oxazolone ring):

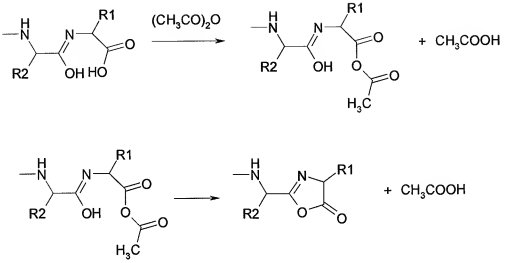
The alkanoic acid anhydride is used as a reagent for formation of the activated C-terminal carboxyl group. The activated C-terminal carboxyl group is reacted with the hydroxyl group to form the 5-oxazolone ring.



(Ib)

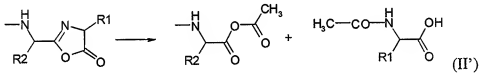
See page 33, line 6 through page 34, line 2 of the specification.

The following is a detailed reaction scheme of the stage (Ib):

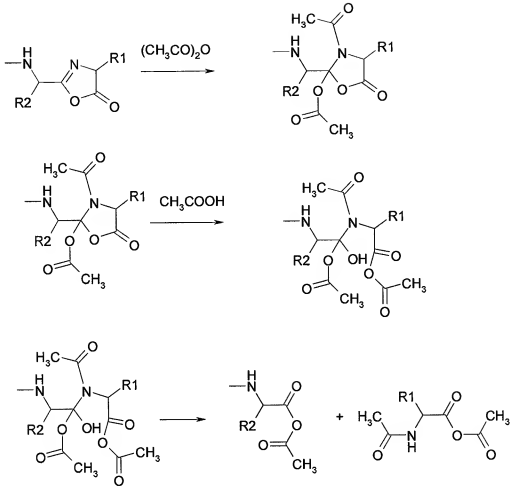


(II') separation of the C-terminal amino acid and formation of the reaction intermediate for the next stage:

The alkanoic acid anhydride is used as a reagent for the addition reaction on the double bond of >C=N- type of the 5-oxazolone ring. The degradation of the 5-oxazolone ring is made via such a reaction as shown by the following reaction scheme (II'):

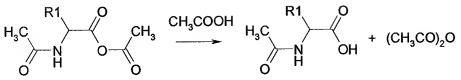


The following may be a detailed reaction scheme of the stage (II').



The alkanoic acid, which is a by-product from the alkanoic acid anhydride formed at the stage (1a) is used as a reagent at the second reaction for opening of the ester bond therein. See page 40, line 7 through page 41, line 4 of the specification.

In addition, the alkanoic acid also reacts on the derivative of the C-terminal amino acid having acid anhydride form, and thereby the C-terminal acid anhydride form thereof is converted into the C-terminal carboxyl group.



The peptidyl reaction product having the activated C-terminal carboxyl group of the third reaction is just ready for the formation of the 5-oxazolone ring at the next stage.

The considerable variation of the reaction speeds of those stages is successfully used to prepare a mixture comprising the original peptide and the series of peptidyl reaction products produced therefrom.

At least, the reaction schemes used in the process for releasing the C-terminal amino acids successively from the peptide of the present invention are quite different from those used in the process for C-terminal stepwise degradation disclosed in Ref. 1: Tsugita et al.

Regarding Ref. 2: Tsugita et al. 1992

Ref. 2: Tsugita et al. 1992 teaches a reaction of pentafluoropropionic anhydride ($(CF_3CF_2\text{-}CO)_2O$) **vapor** on polypeptide, in which a vapor phase reaction is made with the pentafluoropropionic anhydride ($(CF_3CF_2\text{-}CO)_2O$) vapor at such a low temperature as -18 °C. See the abstract of Ref. 2: Tsugita et al. 1992.

However, Ref. 2: Tsugita et al. 1992 fails to provide any suggestion as to the reaction condition used for the liquid phase reaction used in the process for C-terminal stepwise degradation disclosed in Ref. 1: Tsugita et al.

Regarding Ref. 3: Vogt

Ref. 3: Vogt et al. teaches such a process for preparation of a high reactive gel-suspension of carboxymethyl cellulose (CMC), in which the polymer (carboxymethyl cellulose) is treated in a dipolar-aprotic solvent, such as N,N-dimethylacetamide and dimethylsulfoxide (DMSO), with p-toluene-sulfonic acid. See Abstract of Ref. 3: Vogt et al..

Ref. 3: Vogt et al. also provides such assumed mechanism that the activation (swelling in the dipolar-aprotic solvent) is achieved via an interaction between the carboxylate groups (-CH_2-COONa) of Na-CMC and HO_3S-groups of the p-toluene-sulfonic acid with a rapid exchange of the acidic hydrogen as well as an interaction of the lipophilic toluene unit of the p-toluene-sulfonic acid with the solvent. See paragraph bridging page 552 and 553 of Ref. 3: Vogt et al.

Accordingly, Ref. 3: Vogt et al. fails to teach any process for preparation of gel-suspension of CMC in the dipolar-aprotic solvent without p-toluene-sulfonic acid.

Ref. 3: Vogt et al. also reported that an effective method for activation of CMC is precipitation of an aqueous solution of CMC by N,N-dimethylformamide (DMF) and the removal of the water from the swollen gel by repeated distribution under reduced pressure. This report may indicate that N,N-dimethylformamide (DMF) can never remove water from the water-swollen gel of CMC. See page 549, paragraph 3 of Ref. 3: Vogt et al.

Further, Ref. 3: Vogt et al. reported that other acids like methane sulfonic acid, trifluoroactic acid and monochloroactic acid do not swell CMC to a comparable extent. Furthermore, Ref. 3: Vogt et al. reported that polysaccharides with directly at the polymer backbone bound carboxy groups like sodium alginate, sodium pectinate, and 6-carboxy cellulose do also not swell in the manner described for CMC. See paragraph bridging pages 551-552 of Ref. 3: Vogt et al.

In view of these facts, Ref. 3: Vogt et al. fails to provide any suggestion as to whether or not the combinational use of the dipolar-aprotic solvent with other acid than p-toluene-sulfonic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

At least, Ref. 3: Vogt et al. fails to provide any suggestion as to whether or not the combinational use of the dipolar-aprotic solvent with perfluoroalkanoic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

Further, Ref. 3: Vogt et al. fails to provide any suggestion as to whether or not the use of the dipolar-aprotic solvent without p-toluene-sulfonic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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